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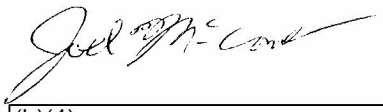

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(ix) BioSpyder grants to the EPA or its authorized representatives, the right to examine, for purposes of verifying the information submitted, those books, records, documents, and other supporting data that will permit adequate evaluation; and this right may be exercised in connection with any such reviews deemed necessary by the Government.

Technical Proposal

I. Technical Approach

1. High Throughput Capacity

Targeted RNA-Seq TempO-Seq assays are carried out in 96- well format as requested by the EPA Contracting Officer or Contracting Officer Representative (COR). Because the assay can accept lysates as input there is no requirement for RNA purification of sample inputs and the samples are processed in 96-well plates through the entire assay and pooled/sequenced. As an addition only assay, TempO-Seq is easily automated using established protocols. Compound treated and untreated RNA or lysate (samples lysed in TempO-Seq lysis buffer) provided by the EPA Contracting Officer or COR are tested when supplied, together with no-sample and positive process controls. BioSpyder will process the samples through the TempO-Seq assay, making targeted RNA-seq libraries directly from the cell lysates, perform sequencing and data analysis, and deliver the data to the EPA Contracting Officer or COR.

The TempO-Seq assay uses a single pair of detector oligos to measure each gene, forming a single ligated probe/gene. The performance of each probe has been validated, and data can be provided to the EPA Contracting Officer or COR upon request. Use of TempO-Seq provides the EPA Contracting Officer or COR the option to request custom attenuation of highly expressed genes. A list of the genes for which attenuation probes have been validated will be provided to the EPA Contracting Officer or COR to facilitate a request for custom attenuation. Once attenuated, this custom attenuated version of the whole transcriptome assay will be available for the EPA Contracting Officer or COR to specify as the assay used for a specified batch of samples, providing the option to use the current whole transcriptome assay or the custom attenuated whole transcriptome assay. Additionally, attenuated pools require less sequencing depth, minimizing the cost and time spent on sequencing. Residual library material not used for sequencing shall be frozen and shipped to the EPA upon the request of the EPA Contracting Officer or COR.

2. Reliable Assays

TempO-Seq is a targeted sequencing assay that provides gene expression data with the sensitivity to measure down to the single-cell level, using either RNA or cell lysates, with the specificity of a single base at the point of ligation and the ability to measure the whole transcriptome. For the proposed program, cells will be lysed and the TempO-Seq assay carried out directly using this lysate. TempO-Seq uses a pair of detector oligos (DOs) that hybridize to target RNAs such that they can be ligated together on the template RNA to generate DNA probes which are then sequenced. Though a ligation-based assay, TempO-Seq is very different from any other ligation-based assay, including RASL-Seq (1,2), incorporating multiple changes that improve performance and provide increased flexibility. TempO-Seq is a capture-free assay, without the 3' bias of other ligation-based assays and requirement that the target RNA have a poly A+ sequence. (b)(4)

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Multiplexed targeted RNA-sequencing of the whole transcriptome of 21,350 protein coding genes and assay of RNA:

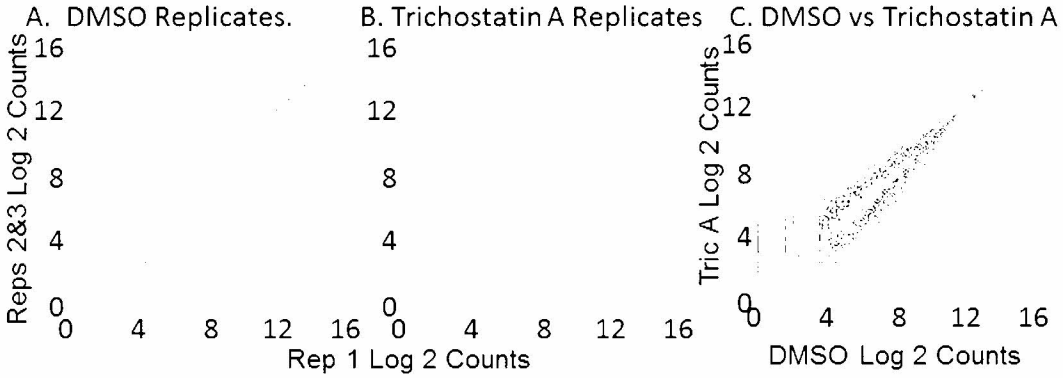

Fig 6: Whole Transcriptome TempO-Seq assay of Cell Lysates. Panel A: Correlation plots of replicates of DMSO control cell lysates. Panel B: Correlation plots of replicates of 1 uM Trichostatin A-treated cell lysates, average of 2.6 M reads/sample. Panel C: Correlation plot of control versus treated, demonstrating differential expression.

Fig 6a depicts the reproducibility between replicates of control cells and cells treated with 1 uM Trichostatin A (Fig 6b) tested as cell lysates in the TempO-Seq whole transcriptome assay, with an average of 2.6 M reads/sample. Fig 6c depicts the differential expression between control and treated cells. The average CV for expressed genes was <10%.

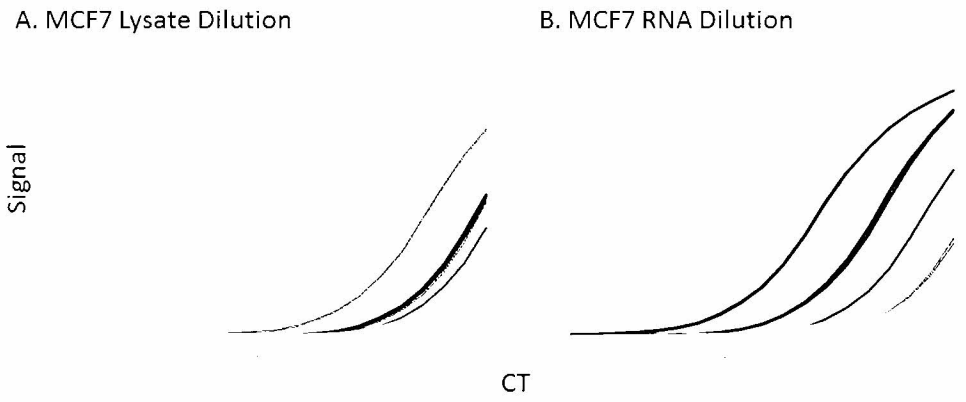

Fig 7: qPCR Tracings from the Whole Transcriptome TempO-Seq assay of Cell Lysates from different Numbers of Cells. The qPCR tracings clearly demonstrate the sensitivity of the TempO-Seq assay. Panel A is lysate from 4,000, 400 and 40 (triplicate) cells, as labeled compared to no sample control. Panel B is that for 100 ng RNA, 10 ng RNA, and 1 ng RNA vs no sample control.

Fig 7 depicts the qPCR tracings from a titration of cell lysates or of RNA to demonstrate the practical sensitivity of the whole transcriptome TempO-Seq assay. From these plots it is apparent that as few as 40 cells or 1 ng RNA can be used as input, though 400 cells and 10 ng RNA is a conservatively robust sample size. The figures depicts use of 25 cycles in this experiment, though

typically the PCR would be carried out to 32 cycles so that the all sample inputs would have saturated, exhausting the primers in the assay. By using 32 cycles each sample is amplified to an endpoint so that samples that vary with respect to input (and are within the acceptable range of inputs), will reach roughly equivalent amounts of amplified product, assuring approximately equal sequencing depth per sample.

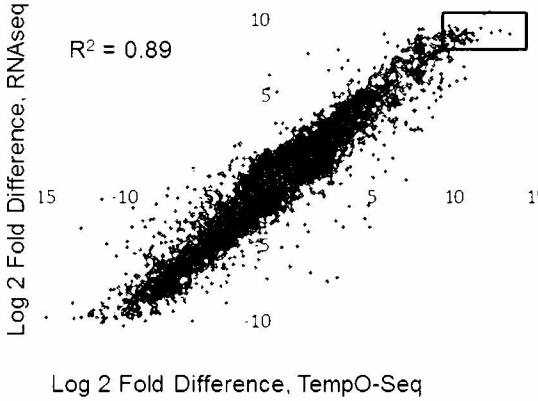

Fig 8: Whole Transcriptome TempO-Seq assay versus RNAseq. MAQC Universal Reference RNA vs MAQC Brain RNA were analyzed by the whole transcriptome human TempO-Seq assay, differential expression was quantified by DESeq, and the fold change detected by TempO-Seq plotted against RNAseq fold changes reported in the MAQC study. The whole transcriptome assay gave an average of ~2000 reads per expressed gene, total 33 M reads, and the RNAseq data had an total of 44 M reads. The data plotted represent the 12,298 differentially expressed s for which at least a base mean of 20 reads were detected in the TempO-Seq assay. Fold changes correlation between TempO-Seq and RNAseq was R^2=0.89. The red box surrounds the genes with the greatest fold change in TempO-Seq assay, from 600 to 12,000 fold change, which in the RNAseq data exhibited instead the same maximal fold change of ~250 to 525, suggesting that the dynamic range in fold change difference of RNAseq was capped.

Multiplexed targeted RNA-sequencing of the whole transcriptome of 21,350 protein coding genes and assay of RNA:

Fig 8 is a correlation plot comparing differentially expressed genes (12,298 were differentially expressed using a cut-off of 20 counts for the TempO-Seq data) identified using the TempO-Seq whole transcriptome data to RNAseq data. The samples used to measure differential expression were the MAQC reference RNA A (Universal Reference RNA) and the MAQC reference RNA B (Brain RNA). RNAseq data were those reported in public databases for these commercial reference RNA samples. The correlation was excellent, R^2=0.89 (r=0.94), better than that reported (3) for the inter-platform correlation of HiSeq to Ion Torrent of r=0.86 or 0.89, and close to the intra-platform correlation for the HiSeq of r=0.98 and 0.99. This plot was generated from TempO-Seq data at an average of ~2000 reads/gene (33 M reads/sample) compared to RNAseq at 44 M reads/sample. This correlation plot also demonstrates the impact of limited RNAseq dynamic range compared to TempO-Seq. As indicated by the genes within the red box, the genes with the greatest differential expression of 600 to 12,000-fold in the TempO-Seq assay were all maxed out at a change of ~525-fold in RNAseq, suggesting that biological differences greater than this cap of ~525 are not accurately measured by RNAseq.

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Fig 10 demonstrates that the TempO-Seq assay can identify 1.2 fold differences at a $p_{adjusted}<0.05$ for genes with >20 counts. These data were generated using the S1500+v3 surrogate assay implemented by NIEHS. As described above, unlike a standard p value, the adjusted p value uses a multiple hypothesis correction (Benjamini-Hochberg) for false positives and is thus more rigorous than the standard p value. It is the norm for microarray and RNA-seq data, hence this is the approach we took. Using the MAQC Reference RNAs, mixtures were made such that differential transcript abundance was fixed at 1.2 fold. Panel A depicts differential expression when comparing Brain vs Brain, a measure of false positives, of which there are 3, consistent with a $p_{adj}<0.05$. Panels B and C show differential genes that are "induced"

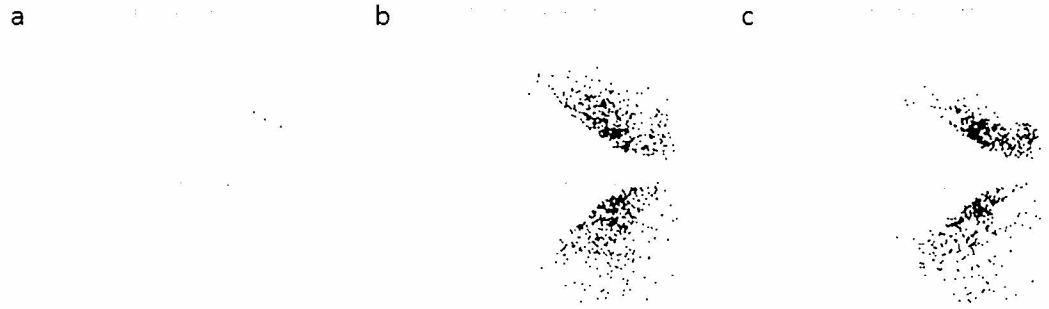

Fig 10: Detection of Differential Expression at a 1.2-Fold Difference in Transcript Abundance. MAQC reference RNAs were mixed in different ratios such that differentially expressed transcripts were 1.2 fold different in abundance. Probes that were differentially expressed between these mixtures at a $p_{adj}<0.05$ are colored red. Panel a illustrates the false positives detected when comparing replicates. Panels b and c show that large numbers of genes are still detectable when abundance differs by 1.2 fold.

(red symbols, upper right quadrant) or "suppressed" at an adjusted p<0.05 when comparing samples that differ 1.2-fold difference in abundance. This result demonstrates that the TempO-Seq assay can readily identify changes in abundance of 20% at an $p_{adjusted}$ <0.05. RNAseq cannot identify differences in expression as small as 1.2-fold.

The TempO-Seq assay is resistant to RNA degradation (Fig 11). RNA was heated to cause degradation, as depicted in Panel A. The correlation of degraded RNA, whether RIN of 7.5 (Panel B) or RIN of 3 (Panel C) to undegraded RNA (RIN of 9.1) was excellent, and there were no genes for which the abundance changed by greater than 2-fold due to RNA degradation.

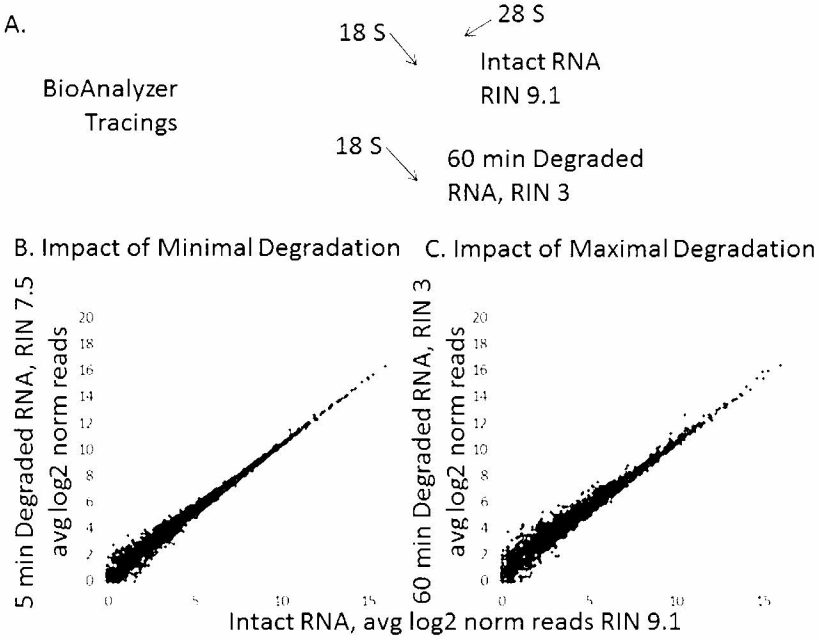

Fig 11. TempO-Seq Resistance to RNA Degradation. Panel A: RNA was degraded by heating at 95°C. Panel B: correlation plot of RNA heated for 5 min (RIN 7.5) to undegraded RNA (RIN 9.1). Panel C: Correlation plot of RNA heated for 60 min (RIN 3) to undegraded RNA (RIN 9.1). No DO exhibited a decrease 2-fold or more due to degradation.

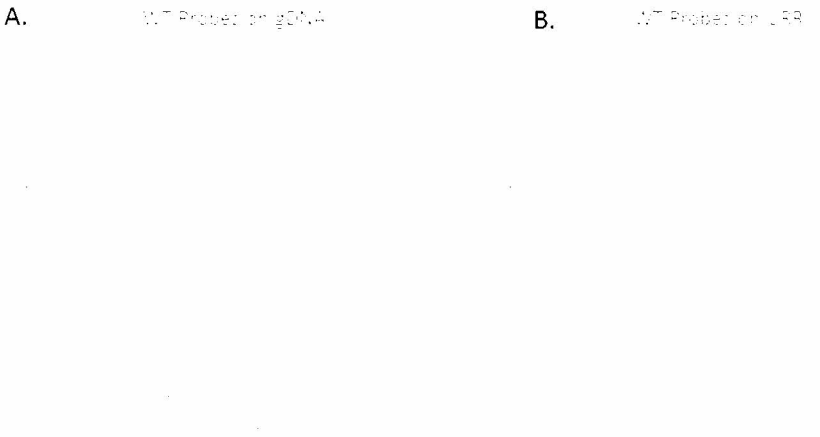

Fig 12. Validation of TempO-Seq Probe Function. Panel A: gDNA was used to validate the function of probes that do not span an exon junction, Over 99% of probes passed this QC. Panel B: QC using MAQC RNA to measure function of the DOs. Those Dos that were not functional against qDNA were functional against the universal reference RNA, and vice versa.

Probe validation and validation of the use of a single probe per gene:

The functional performance of each DO set is validated using gDNA (Fig 12) as well as MAQC universal reference RNA. Panel A depicts the function of DOs on qDNA. Over 99% were functional. To confirm that the remainder of the DOs were functional and independently confirm the functionality of most of the other DOs we also tested against the MAQC reference RNA (Panel B). Those DOs that failed to show function on gDNA were functional on RNA. Testing on both gDNA and RNA is the end of an iterative process of probe refinement. Any

probes that failed to show signal in initial QC tests on both qDNA and RNA are replaced with DOs measuring the same gene targeting a different sequence of that gene.

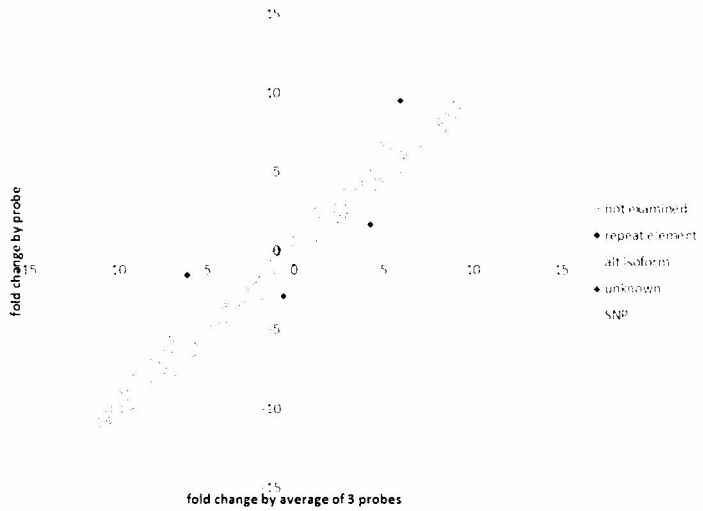

Fig 13. No difference between 3 probes per gene vs 1 probe per gene. Fold difference in expression between HepG2 and MCF7 cell lysates were measured in an assay using 3 probes per gene. The plot depicts the fold change measured by each probe alone versus the average of all three probes. The correlation was R^2=0.977 for all probes. Analysis of the outliers identified 2 that targeted repeat sequences, 2 targeted SNP regions, 3 alternate isoforms, and 1 unknown. With removal of the outliers R^2=0.983.

A single DO pair per gene is sufficient to measure the presence and amount of each RNA (Fig 13), reducing the complexity of the library to just these (50-base long) probes. This was carried out early in the development of the assay, and the DO design software was modified to assure that there were no probes that targeted regions of known SNPs, or repeat sequences, and the isoforms targeted by each probe are defined.

Absolute sensitivity:

The absolute sensitivity of the TempO-Seq assay was determined using the External RNA Controls Consortium (ERCC) reference RNA (Ambion ERCC RNA spike-in control Mixes 1 and 2). These were titrated in a background of 50 ng/µL MAQC Reference RNA and assayed using a DO cocktail for the ERCC target sequences. The ERCC RNAs are synthetic reference RNAs that are not present naturally and are supplied in a cocktail containing a range of abundance. Fig 14 presents the regression plots of the abundance of each RNA species in the cocktail vs counts at a dilutions of 1 x 10^{-3}, 1 x 10^{-4}, 1 x 10^{-5}, and 1 x 10^{-6} for Mix 1 and Mix 2. (b)(4)

(b)(4)

A B

Fig 14: ERCC RNA Regression Plots of Counts vs Input Abundance. ERCC synthetic RNAs were initially diluted by 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, to bring their range close to the limit of detection. Panel A depicts the TempO-Seq results for different known amounts of RNAs in Mix 1, and panel B shows the same for Mix 2. (b)(4)

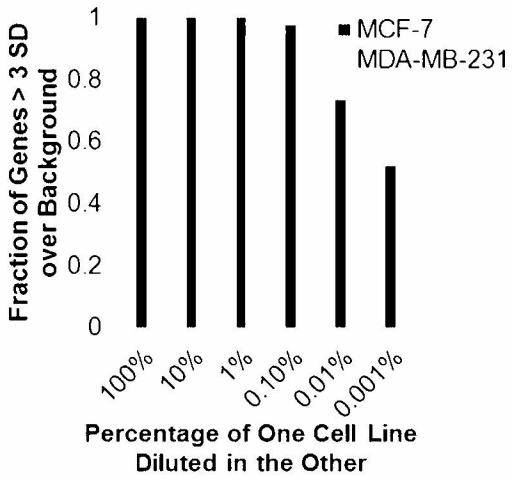

Fig 15: Detection of One Cell in 100,000. MCF7 cells were diluted serially in MDA-MB-231 cells (dark green) or MDA were diluted serially in MCF7 cells (light green), then lysed. The horizontal axis indicates the percentage of the minor cell line in the lysate, and the vertical axis the fraction of MCF7-specific or MDA-specific genes detected above background + 3SD. Results indicate the ability to detect 1 in 100,000 cells.

An independent method was used to corroborate this absolute sensitivity using cell lysates. MCF7 cells and MDA231 cells were serially diluted into a constant number of the other cell type each other, then lysed the mixtures were profiled to identify the specific 14 or 13 genes expressed in MCF7 or MDA231 cells, respectively. Fig 15 shows that cell-specific transcripts from the minor cell type were still detectable in a dilution of 1 cell in a background of 100,000 cells. A set of 7 MCF7 genes reported to have an abundance from 4 to 2,200 copies/cell gave counts of 3 to 185 above background (average plus 3SD, indicating that the assay was detecting as few as ~10 transcripts in cell lysates, consistent with the ERCC results, but demonstrating this absolute sensitivity is also a practical sensitivity.

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Sample Barcoding, Sequence Alignment, and Analysis:

As depicted in Fig 1, dual index sample barcoding is used, compatible with the (b)(4) (b)(4) software of Illumina sequencers. A set of proprietary barcodes is used. The

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sequencer serves as a counter, using the facility provided by the sequencer software to sort data by barcode into sample-specific output (providing a FASTQ output file for each sample). (b)(4)

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After the data are demultiplexed into separate FASTQ files for each sample by the Illumina sequencer using the (b)(4) software and the standard dual indexing Illumina sample sheet and BioSpyder index sequences, the FASTQ files are aligned to a look-up table "genome" of the known probe sequences comprising the cocktail used for the whole transcriptome assay using the ultrafast short read aligner Bowtie and multiple processors to align samples in parallel, generating a table of counts with each column representing a sample and each row representing a probe. QC metrics are calculated, such as alignment efficiency, determination of mapped and unmapped reads (Fig 20), sample clustering to identify that replicate samples used to derive averages, statistics, (b)(4)

(b)(4) and assessment of replicate performance. This is output as a sequencing data quality report. (b)(4)

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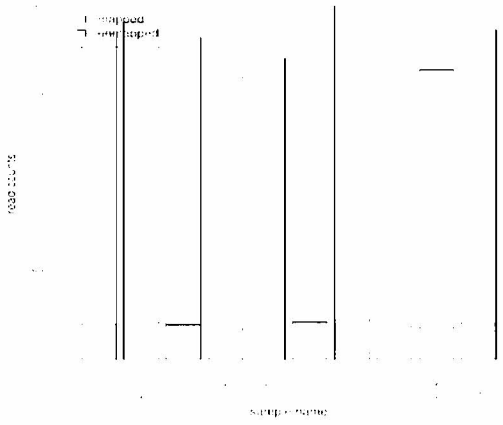

Fig 20: Mapped vs Unmapped Reads Across QC Samples. Total reads per sample are aligned to the expected ligated sequences. Aligned (mapped) reads are shown as a fraction of the total reads.

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Table 1 shows a set of exemplar genes selected from an assay depicting how the output file is organized.

Table 1: Example Data Output File

Probe_ID	RNA Input Universal Ref RNA 100 ng rep 1	RNA Input Universal Ref RNA 100 ng rep 2	RNA Input Universal Ref RNA 100 ng rep 3	RNA Input Universal Ref RNA 10 ng rep 1	RNA Input Universal Ref RNA 10 ng rep 2
RPS14_16014	938	1077	1338	1109	918
RPS15_5978	476	461	620	373	281
RPS15A_5979	77	78	71	58	61
RPS16_15091	990	831	1211	804	594
RPS17_5980	0	0	0	0	0
RPS17L_5981	13336	11636	17497	8056	6336
RPS18_5982	8322	6877	9957	4891	3981
RPS19_15089	6152	5470	7978	4311	3371
RPS19BP1_15609	130	135	204	109	79
RPS2_5983	502	574	683	436	351
RPS20_27810	330	273	404	290	242
RPS20_5987	1	5	1	0	2
RPS21_5988	337	340	436	253	226
RPS23_27811	450	519	702	365	311

The first column lists the gene names, indicating the specific probe used by a unique extension that indicated the probe sequence. The successive columns are the counts for each gene for each sample, in this case triplicate samples of gDNA, HepG2 cells, MCF7 cells, or no sample control (water).

Within the software, normalization options are offered, and subsequently, the software can compare treatments and identify differentially expressed genes. Normalization options include:

- Normalization to total counts (b)(4)
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- Normalization by “size factor”, which is similar to normalizing by total counts but calculates a geometric mean for each gene across all samples, generates a “reference” sample comprised of these geometric means, then for each sample calculates a quotient of counts/reference and a median as the size factor for each sample which is then used for normalization – thus (b)(4)

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- Normalization to designated housekeeping genes.
- *De novo* identification of stable genes (those with low CV between treatments) and using these stable genes for normalization.

Table 2: Example Data Output File

Gene	baseMean	log2FoldC	lfcSE	stat	pvalue	pad	CT_1	CT_2	CT_3	CT_4	CT_5	CT_6	RX1	RX2	RX3	RX4	RX5
DHRS2_15	25925.47	4.827209	0.139816	30.94936	2.59E-210	3.93E-206	2209.814	2637.112	2515.511	3003.616	3274.416	1983.194	43964.05	60660.51	51310.71	57069.32	56551.97
RPL3L_122	520.8359	7.167513	0.259269	27.64508	3.20E-168	2.43E-164	5.530679	11.20037	4.516178	2.916132	12.35635	3.304222	1304.283	1217.578	960.9341	840.1775	1366.138
DENND2C	1261.369	2.999698	0.110361	27.18071	1.16E-162	5.55E-159	261.7855	318.594	302.5839	312.9962	312.5135	232.8414	2174.399	2572.375	2228.969	2635.311	2472.793
SAT1_610	9615.532	2.492927	0.103125	24.17382	4.20E-129	1.59E-125	3138.968	3236.965	3053.689	3460.477	2862.15	2688.976	14997.02	18024.02	18438.84	18026.82	17643.92
SPDEF_67	3997.946	-3.25064	0.134549	-24.1595	5.94E-129	1.80E-125	6077.002	5300.651	6505.554	8134.065	4957.664	5690.532	610.6861	610.7217	717.8254	563.1791	678.9222
BATF_246	1070.424	-3.80079	0.156651	-24.1191	1.67E-128	8.93E-125	2171.099	1875.473	1649.91	1596.096	1864.66	2022.184	189.0007	156.5457	176.3445	163.8719	201.4284
ASCL2_17	961.2885	-3.4777	0.145503	-23.9008	3.00E-126	6.51E-123	1549.203	1372.984	1522.705	1790.503	1376.315	1773.046	155.1232	108.2292	162.4664	120.8999	164.6949
DKK3_261	6480.527	2.478059	0.105856	23.40676	9.86E-109	1.76E-121	648.0374	948.815	761.1909	668.4484	631.7004	849.1462	7043.54	6796.618	5489.678	6478.523	6809.364
WSL1_239	826.9143	-3.32846	0.142757	-23.3156	3.03E-120	5.19E-117	1377.754	1134.991	1505.393	1213.111	1602.329	1584.705	148.8826	123.6905	145.4765	127.0214	132.7038
DHRS2_18	5699.296	4.146841	0.178585	23.1948	5.14E-119	7.80E-116	514.7886	893.2645	537.4251	590.0307	479.2339	859.0478	14101.95	8743.369	10625.09	8625.01	10760.85
TSKU_737	2550.618	3.66053	0.159974	22.8821	7.01E-116	9.66E-113	4667.279	4518.308	4854.591	5514.406	3546.471	3272.502	284.3925	396.196	295.2204	230.7718	415.8843
HBEGF_15	898.1923	2.263778	0.122096	22.63617	1.91E-113	2.41E-110	290.0834	219.0334	219.7873	258.3637	279.5021	244.5125	1579.836	1903.674	1766.955	1658.93	1559.27
WIBG_190	4311.03	2.45532	0.108697	22.5887	5.60E-113	6.53E-110	7339.211	7067.56	6957.172	7107.586	6835.536	5909.932	1112.608	1337.403	1276.37	1338.765	1079.403
DKK1_252	4246.104	-3.05374	0.135654	-22.5113	3.22E-112	3.49E-109	7765.688	7137.253	6107.578	6222.054	7667.045	7627.467	1064.466	933.4764	750.7434	762.1282	669.4433
CDK5_240	703.6909	3.041022	0.136189	22.32937	1.92E-110	1.94E-107	146.2557	169.2531	167.8513	169.1337	193.9258	122.2562	1202.65	1481.43	1319.907	1329.898	1468.036
PGF_5091	442.5092	4.108944	0.188809	21.76224	1.49E-105	1.41E-102	43.63091	64.71441	62.47379	45.68607	47.43509	38.98982	584.8322	977.9277	1062.934	973.3204	965.6572
CHRV4_2	219.0486	5.078827	0.238562	21.74564	7.70E-105	6.87E-102	15.368	8.711555	9.785052	7.776852	19.25392	17.18196	388.7748	579.799	499.0798	505.0247	415.8843
KCNN2_21	175.9939	6.033976	0.286176	21.10231	7.57E-99	6.38E-96	4.916159	0	2.258038	7.776852	5.580599	6.604445	497.464	359.4754	332.3659	384.8375	324.6504
ANKRD46	537.8484	4.777644	0.228574	20.90197	5.14E-97	4.19E-94	41.14286	42.81327	45.66717	44.71403	35.57632	17.18196	624.4079	1291.019	1067.181	1288.578	1418.593
SGTB_107	1073.619	2.737432	0.133162	20.55719	6.64E-94	5.04E-91	272.8468	349.7267	230.7557	268.2842	358.5535	246.495	1789.266	2009.97	1843.42	2101.209	2291.51
FOXA1_22	7420.614	-2.15973	0.105488	-20.469	4.07E-93	2.94E-90	1112.014	12875.68	10258.5	10965.64	11640.43	11942.71	2236.73	2475.742	2259.292	2685.813	2672.09
RGS2_582	466.0045	3.681543	0.184559	19.90473	3.70E-88	2.35E-85	58.99391	67.20343	57.20492	118.5894	55.10341	68.06638	840.6964	983.7257	880.2918	1115.646	913.3236
DYRK3_20	1034.625	3.313879	0.167708	19.75965	9.60E-87	4.35E-84	221.8417	199.1213	195.201	247.8712	113.7047	211.4702	1759.846	1787.714	2073.836	2086.486	2085.586
PAT21_27	584.8808	3.6762	0.186334	19.7292	1.21E-86	7.66E-84	1000.438	1066.343	1126.796	1161.593	978.9072	726.9283	64.1889	75.37387	112.5534	68.86701	84.50335
C9orf69_2	1960.279	-2.56837	0.131101	-19.5908	1.85E-85	1.12E-82	2935.952	2598.533	3563.264	3760.838	2910.282	3119.136	431.4921	583.6644	488.4611	555.5879	562.8663
SAT1_175	4118.978	2.641147	0.135316	19.51811	7.70E-85	4.50E-82	1009.042	1301.755	1330.415	1300.595	1307.255	903.3569	7732.98	8161.638	8212.517	7884.507	8112.669
CTDSP1_2	1937.673	-2.3366	0.119755	-19.5115	8.77E-85	4.93E-82	2907.294	2806.365	3207.239	3312.726	3269.533	2321.606	526.8839	562.4051	645.6181	728.4599	526.6758
ACSBG1_2	231.8217	6.222456	0.31895	19.50917	9.18E-85	4.97E-82	5.530679	4.978032	5.266874	11.66488	0	4.629911	548.2802	400.0618	321.7472	457.583	790.2986

The software will perform a simple analysis of normalized data to identify differentially expressed genes (or modulated genes in the case of comparison of treated to untreated cells). (b)(4)

(b)(4) Tables 1 and 2 depict the type of data generated. This is Trichostatin A data for MCF-7 and MDA-MB-231 treated cells and using universal RNA reference samples as controls. These data also included platform comparison data. Table 1 is raw data, Table 2 (supplementary files) is processed data.

3. Laboratory Practices

BioSpyder has a Quality Management Plan in place which passed an audit by the EPA in 2019. The QA manager is the CSO, (b)(4) who monitors the assay, manufacturing, and probe design/data analysis processes, and reviews data before they are delivered to customers. The organization of BioSpyder assures independence of QA. Dr. Yeakley reports directly to the CEO, Joel McComb, as does (b)(4) The BioSpyder employees that will conduct this work report to (b)(4) (b)(4)

(b)(4)

(b)(4) All staff have access to the President, either in person, by phone, or by other electronic means.

The BioSpyder Technologies' Quality System consists of a Quality Management Plan, project-specific QA Project Plans, and written SOPs (b)(4)

(b)(4)

Training in SOP's is done by reviewing work processes with those doing manufacturing, assay processing, and data analysis. Evidence of adequate training is ensured by observation of processes and inspection of manufacturing travelers, functional testing of manufactured reagents using standard samples in the assay, and inspection of resulting data for standard samples.

Service projects are planned by writing a Project Plan that summarizes the genes monitored, the samples assessed, with sample layouts (including control samples), and the data to be delivered, with expected performance metrics for passing results. The TempO-Seq assay can be monitored for product yield in real time, so samples with low yields (or problematic input quality) can be identified independently of the final sequencing output, which is assessed for performance metrics, with acceptance criteria for control samples and overall performance defined in the Project Plan.

The QA manager has responsibility for reviewing reports to assure they met SOP standards and QA standards before the report and data are sent to the client.

The Project Plan will include quantitative criteria for the decision to pass or fail test data for each assay on a per plate basis. The QC data shall be provided along with the results from analysis of each sample order/batch.

II. Technical Qualifications, Education, Experience, and Expertise of Key Personnel

- Program Manager – (b)(4) (b)(4)

(b)(4) is a co-founder of BioSpyder Technologies and currently serves as VP of Research and Development. She oversees the development program and the execution of projects supported by grants and contracts. [redacted] (b)(4) manages experimental planning and troubleshooting, directs research personnel, collaborates with contractors and customers, and oversees product manufacturing and service projects. (b)(4) (b)(4)

Prior to BioSpyder [redacted] (b)(4) held various positions at Illumina from 2001-2011. She was a key member of a team that developed a gene expression assay for measuring transcripts in degraded RNA for detection on an Illumina microarray, and she led product development projects in Molecular Diagnostics, including the first team to take an Illumina product through FDA 510(k) clearance. [redacted] earned a (b)(4) (b)(4) (b)(4)

- Senior Laboratory Scientist – (b)(4)

(b)(4) is the Services and Lab Manager at BioSpyder Technologies. She oversees the processing of all samples received by BioSpyder (b)(4) primary tasks include receiving and storing samples, creating and designing project plans, physically processing samples, and generating QC metrics/reports. (b)(4) works closely with (b)(4) to process any troubleshooting requests. During sample processing of calls under (b)(4) [redacted] (b)(4) was able to provide 100% of weekly hours to the project and can continue to do so for future calls. [redacted] (b)(4) worked on samples and managed processing (b)(4) (b)(4) (b)(4) (b)(4)

- Bioinformatics Scientist – (b)(4)

(b)(4) is the director of Bioinformatics at BioSpyder Technologies. He leads a bioinformatics team responsible for building and maintaining TempO-SeqR, a software pipeline (b)(4) (b)(4) (b)(4) (b)(4) (b)(4) (b)(4) (b)(4) (b)(4) (b)(4) worked with BioSpyder staff to generate and perform quality assessments of sequencing data as well as work with EPA personnel to answer questions, troubleshoot, and build custom gene manifests for ease of use.

III. Past Performance

(a) (b)(4)

- (b) EPA-BPA-16-D-003
- (d) Blanket Purchase Agreement
- (e) High Throughput transcriptomics work
- (f) $10,000,000
- (g) 2017-2020
- (h) Kimberly F. Loesch, Loesch.Kimberly@epa.gov
- (i) Joshua Harrill, harrill.joshua@epa.gov
- (b)(4)
- (b)(4)

(b)(4)

(b)(4)

(b)(4)